

FOURTH REPORT OF THE WELLCOME TROPICAL
RESEARCH LABORATORIES, KHARTOUM

VOLUME A - MEDICAL - (B)Pathological Report

1911

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FOURTH REPORT OF THE WELLCOME TROPICAL RESEARCH LABORATORIES AT THE GORDON MEMORIAL COLLEGE KHARTOUM VOLUME A.—Medical

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KALA-AZAR COMMISSION

TO INVESTIGATE THE PREVALENCE AND CAUSE OF THE DISEASE IN THE EASTERN SUDAN

(2) PATHOLOGICAL REPORT

BY

LIEUTENANT W. E. MARSHALL, M.B., Ch. B., D.P.H., R.A.M.C.

Attached Egyptian Army

CLINICAL NOTES

The material for carrying out the pathological investigation into kala-azar in Sennar Province was obtained for the most part from the 12 cases of kala-azar admitted into Singa Hospital between the months of December, 1909, and July, 1910.

The clinical histories of these cases were briefly as follows:—

- Case 1.* A.A.B., boy, aged 10. Admitted into hospital on April 19, 1910, with enlargement of the spleen, irregular fever, and an œdematous swelling of the right cheek. His condition remained the same till May 21, 1910, when the fever became more irregular, jaundice set in, and he died suddenly on June 20, 1910. He had a progressive leucopenia, the white blood corpuscles numbering 5200 on April 24, 1910; 4531 on May 3, 1910; 4375 on May 14, 1910; and 2200 on May 26, 1910. Leishman-Donovan parasites were found in the peripheral blood and by spleen puncture. Post mortem examination showed enormous enlargement of the spleen with white patches on the capsule and some adhesions at the upper end. The liver was also enlarged. The bone-marrow was red but the intestines appeared healthy. Treated with Tinct. Senega.
- Case 2.* A.S.W.O., boy, aged 14. Admitted into hospital on Dec. 7, 1909, with enlargement of the spleen, fever and emaciation. He had had occasional rigors before admission. He became deaf without associated pain, and suffered from night terrors. His temperature was very high and very irregular, and he died on Jan. 26, 1910. The leucocytes numbered 4500. Leishman-Donovan parasites were found in the peripheral blood and by spleen puncture. Treated with tincture of senega and quinine.
- Case 3.* A.W.T., boy, aged 10. Admitted into hospital on Nov. 30, 1909, with splenomegaly, constant fever, and œdema of the feet. He did fairly well, and had periods of apyrexia, the temperature sometimes remaining normal for four clear days. He went home on April 23, 1910, but died there on May 16, 1910. He had albuminuria and also suffered from tapeworm, the ova of *Tænia nana* and *Tænia solium* being both present in the fæces. Leishman-Donovan bodies were found in the peripheral blood and by spleen puncture. Treated with tincture of senega and quinine.
- Case 4.* F.B.A., girl, aged 13. Admitted into hospital on Dec. 7, 1909, suffering from diarrhœa, splenomegaly, fever and emaciation. She had attacks of epistaxis, and suffered from suppuration in the middle ear. She died suddenly on January 9, 1910. Leishman-Donovan bodies were found in the peripheral blood and by spleen puncture. Treated with tincture of senega.
- Case 5.* S.K.W.T., boy, aged 12. Admitted into hospital on Dec. 1, 1909, with fever, swelling of face and feet, and splenic pain but no splenic enlargement. History of rigors every second day at 4 in the afternoon. Leucocytes 5859. He died suddenly on Dec. 10, 1909, complaining of severe pain in the abdomen and chest. Leishman-Donovan bodies were found by spleen puncture, and in the peripheral blood. Treated with tincture of senega and quinine.
- Case 6.* A.A.K., boy, age about 12. Admitted into hospital on Dec. 1, 1909, with fever, splenomegaly, slight enlargement of the liver, and leucopenia, the white blood cells numbering 3125. After a short stay in hospital he was allowed to attend as an

Clinical notes

Clinical notes

- out-patient and steadily improved. On Feb. 27, 1910, he was able to return to school and on April 25, 1910, the splenic enlargement had disappeared. Leishman-Donovan bodies were found in the peripheral blood. Treated with tincture of senega.
- Case 7.* Z.B.B., girl, aged 11. Admitted into hospital on Dec. 7, 1909, with enormous enlargement of the spleen, fever, extreme emaciation and pigmentation of the skin. Liver slightly enlarged. Died on Dec. 19, 1909. Leishman-Donovan parasites found in the peripheral blood. Treated with tincture of senega.
- Case 8.* A.W.B., boy, aged 10. Admitted into hospital on Dec. 11, 1909, suffering from fever, enormous enlargement of the spleen, and œdema of the face. Died on Dec. 17, 1909, six days after admission. Leishman-Donovan parasites found in the peripheral blood and in the spleen. Treated with tincture of senega.
- Case 9.* G.E.S.M., boy, aged 11. Admitted into hospital on Dec. 13, 1909, with enormous splenomegaly, fever, œdema of the feet and face, and slight emaciation. Died on Jan. 5, 1910. Leishman-Donovan parasites found in the peripheral blood and in the spleen. Treated with tincture of senega.
- Case 10.* Y.E.T., boy, aged 10. Admitted into hospital on July 26, 1910, with splenomegaly, fever, œdema of face and feet, and cough with blood-stained expectoration. He died on the day of admission. Leishman-Donovan parasites found in the peripheral blood and by spleen puncture.
- Case 11.* Y.K., boy, aged 19. Admitted into hospital on July 25, 1910, with splenomegaly and slight fever. He also complained of splenic pain. Two days after admission he complained of severe pain in the abdomen, and his temperature rose. The pain continued, diarrhœa set in and he died on July 31, 1910. Leishman-Donovan parasites found in the peripheral blood and in the spleen. Treated with tincture of senega. Post mortem examination showed acute general peritonitis from rupture of an ulcer in the upper part of the descending colon.
- Case 12.* M.E., boy, age about 12. Admitted into hospital on May 31, 1910, with high fever, splenomegaly, œdema of the feet and face, and slight emaciation. He died on June 23, 1910. Leishman-Donovan bodies found by splenic puncture but not in the peripheral blood. Treated with tincture of senega.

Pathological material was also sent by Captain Thomson from cases seen by him which were not admitted into Singa Hospital. The outstanding clinical features of the disease in this Province are splenomegaly, fever, and œdema of the face and feet. With the exception of Case 7, the emaciation was usually slight, this being probably accounted for by the comparatively short duration of the illness. Dyspnœa was a common terminal symptom. Diarrhœa was present in two cases, epistaxis in one, and one died from rupture of an ulcer in the descending colon. One case suffered from extreme deafness and another from middle ear disease. (We have since seen in Khartoum Military Hospital a boy with kala-azar who also had suppuration in the middle ear.) Splenic pain was frequently complained of and death was often sudden. Two cases had rigors. The fever was usually irregular, the maximum rise occurring about 4 in the afternoon.

Treatment. In view of Major Ensor's favourable result with Tinct. Senega this drug was tried in the treatment of the disease. It was given in doses of $\frac{1}{2}$ to 1 drachm 3 times a day. When rigors were or had been present, quinine was also given. One of the cases, No. 6, has apparently recovered, and senega was the only drug used. In another case (Case 3) there appeared to be a marked diminution in the number of parasites found by spleen puncture, and in the same case the flagellated bodies found in the fœces, and to be described later, disappeared after its administration and reappeared again when it was stopped, so it would seem to have a definite toxic effect on protozoa.

Presence of
Leishman-
Donovan
bodies in the
peripheral
blood

PERIPHERAL BLOOD

(a) *Presence of Leishman-Donovan bodies in the peripheral blood*

Out of 15 cases of kala-azar in which the peripheral blood was examined the parasites were present in 13 or in 86.6 per cent. In one of the negative cases only 4 slides were

available for examination. Out of these 13 cases, in 9 the parasites were present in the large mononuclear cells only, in 3 they were found only in the polynuclear cells, and in one case in both. They were never found in very large numbers seldom averaging one parasite per slide. We have found as many as 4 inside one large mononuclear cell. There seems to be a definite relation between the number of parasites in the blood and the number in the spleen. In the negative cases the parasites were in small numbers in the spleen and in Case 2, where the parasites were also scanty in the spleen, they were not found in the peripheral blood until the eighth film had been examined. In the majority of positive cases, however, the examination of 3 slides is usually sufficient for diagnostic purposes. The parasites did not appear to vary with the time of day nor with the fever, nor could we find them markedly increased before death.

(b) *Increase in the large mononuclear leucocytes*

This as a rule was well marked, varying from 8.19 per cent. to 38.21 per cent. of the white cells. The case with the lowest percentage of these cells was the one which recovered. There was frequently marked vacuolation of these mononuclear cells and some were comparatively of an enormous size, one measuring 34μ by 39μ being found on one occasion.

Increase
in the large
mononuclear
leucocytes

(c) *Other blood changes*

Except in Case 6 there was a definite diminution in the number of polynuclear cells. Large deeply basophile mononuclear cells were frequently present. These, in our opinion, are plasma cells. The nucleus is excentrically placed, there are small vacuoles in the protoplasm, and the perinuclear zone, so characteristic of these cells, is well marked. The wheel-shaped structure of the nucleus was absent, but this does not occur, according to Naegeli, when these cells are present in the peripheral blood. It is difficult, however, in the peripheral blood to distinguish the above from Naegeli's myeloblasts. Nucleated red cells were occasionally found and megalocytes were common. Polychromatophilia was often well marked.

(d) *Relation of eosinophilia to kala-azar*

In well-marked cases of kala-azar there is undoubtedly a diminution in the number of eosinophile cells present in the peripheral blood. It is interesting to note, however, that a great many cases of fever and splenomegaly suffered from eosinophilia. Out of 43 such cases where the peripheral blood was examined 18 showed eosinophilia, 2 showed eosinophilia and quartan malaria parasites, 2 showed quartan malaria only, and 21 showed no special change. Another interesting fact is this:—2 cases of kala-azar diagnosed positive by Captain Bousfield, and 2 cases put down by him as suspicious (one, No. 6 of this series) were seen by us. The first two were both healthy. One (S.M.) had an eosinophilia of 8.82 per cent., and in the other (R.S.) the eosinophilia is noted as "enormous." Of the two suspicious cases one (G.S.) showed definite eosinophilia in the peripheral blood; the other (No. 6 of this series) had, when first examined, an eosinophilia of 2.86 per cent., but after recovery he also showed an increase in the eosinophile cells. The brother of Case 2, who had splenomegaly, also showed eosinophilia in the peripheral blood and a local eosinophilia in the spleen. The sister of Case 6, suffering from fever and splenic enlargement, had also eosinophilia in the peripheral blood. Two Abyssinians were admitted into Singa Hospital suspected to have kala-azar. One, Case, No. 11, was proved positive; the other, whose symptoms suggested the disease, has

Relation of
eosinophilia
to kala-azar

so far shown no parasites in the spleen or peripheral blood and is still under observation in Singa Hospital. Eight more Abyssinians lived with the above and all were examined. One had an enormous spleen but the others appeared healthy. The peripheral blood was examined in all cases with the following results:—

No. 1	...	...	...	11.1 per cent. eosinophiles
„ 2	...	...	...	10 „ „
„ 3	...	...	...	30 „ „
„ 4	...	...	...	3.2 „ „
„ 5	...	...	...	2.7 „ „
„ 6	...	...	...	8 „ „
„ 7	...	...	...	33.3 „ „
„ 8	...	...	...	13.7 „ „

URINE AND FÆCES IN KALA-AZAR

Flagellated
bodies in fæces

Examination of these was carried out in order to determine, if possible, if the kala-azar parasite leaves the body with the excreta. In the urine, albumin was twice present, but microscopical examination failed to reveal anything abnormal. In the fæces no kala-azar parasites were found. In 4 of the 8 cases examined, however, flagellated bodies were present in the fæces, in 3 of them in enormous numbers. In hanging-drop preparations these are seen as actively motile bodies darting backwards and forwards across the field. As they gradually lose their motility they become actively amœboid and throw out long narrow pseudopodia. In specimens stained by Leishman's method they appear as oval bodies varying greatly in size but averaging about 13μ by 11μ . They appear to have 4 flagella (sometimes only 3 can be seen) coming off near the nucleus, and one flagellum is longer than the others (Plate X., figs. 1–4). In one of the small forms, measuring 5.9μ by 10.2μ , the longest flagellum measured 13.6μ in length, and in another similar parasite the flagellum measured 20μ in length. The protoplasm is vacuolated and there appear to be bacteria, etc., in the interior of the parasite. They remain alive and actively motile at 22° C. for at least 6 days. They were best found in the fluid portion of the stool after an aperient had been administered. If not originally present, cultivation at 22° C. did not show any parasites.

In Case 3, the ova of *Tænia nana* were constantly present in the stools and the ova of *Tænia solium* were also found on two different occasions. Actively motile spirochætes were also frequently found.

Somewhat
similar bodies
found in
healthy
controls

In order to determine if these flagellated bodies are common in the alimentary canal of the inhabitants, numerous controls, numbering 35, were examined. Twenty of these were school-boys attending school, 3 were apparently healthy young children, and the remainder were patients sick with fever and other illnesses. Flagellated bodies resembling the above were found in 5 cases but always in small numbers. They appeared similar to the above but they were present in such small numbers that microscopical examination of stained specimens was impossible. Three of the positive cases were school-boys; No. 1, Spleen normal, no fever, quartan malaria and eosinophilia in his blood; No. 2, Spleen normal, no parasites in peripheral blood; No. 3, Spleen enlarged, no fever, quartan malaria and eosinophilia in the peripheral blood. Of the other two, one was a woman admitted

into hospital with quartan malaria and slight splenomegaly, while the other was a child, an out-patient with diarrhœa. Out of these 35 cases the ova of *Tœnia nana* were found in 4 cases—all school-boys—and the ova of *Fasciola ægyptica* in one case of fever and splenomegaly.

The presence of four free flagella and the absence of an undulating membrane would indicate that these bodies are trichomastix forms of trichomonas. The flagella usually arise from the blunt end of the parasite, but sometimes from the side. The vacuolation of the protoplasm, so well marked in the larger forms, is usually indistinct or absent in the smaller forms of the parasite. Bodies resembling the so-called cysts of trichomonas were also present along with these trichomastix bodies and are shown in Plate X., figs. 1-2.

Flagellated bodies have been frequently found in the human alimentary canal, and are generally supposed to be harmless inhabitants of that canal. Castellani thought that, though their presence in small numbers might be harmless, their presence in large numbers was the ætiological factor in two cases of diarrhœa in Ceylon. Still more recently Castellani and Chalmers have found another flagellate in the fæces in cases of diarrhœa. It has two flagella and an undulating membrane and has been called by them *Bodo asiaticus*. Wenyon has recently found a new flagellate, *Macrostoma mesnili*, in the fæces of a native of the Bahamas. It has three flagella and a large cytostome. It appeared to have no ill-effects upon the host.

Previous notes
of flagellates
in fæces

Cummins noted the presence of flagellated bodies in the fæces of a case of kala-azar occurring in the Sudan. He also stated that he had seen similar bodies in a case of acute dysentery.

A young dog was fed frequently with milk mixed with the fæces of kala-azar patients, sometimes with and sometimes without flagellated bodies. It remained perfectly well and was chloroformed 54 days after the first feeding. There was no evidence of infection with kala-azar.

THE PRESENCE OF LEISHMAN-DONOVAN BODIES IN DOGS AND OTHER ANIMALS

In view of Nicolle's discovery of spontaneous Leishmaniosis in the dog, recently confirmed by Gabbi and Basile in Italy, Critien in Malta, Alvarez in Lisbon, and the Sergents in Algiers, 21 dogs from the village of Singa were killed and examined. Nine of these were emaciated but showed no symptoms of illness, 2 were emaciated and ill, and 10 were normal. One of the latter was from a *tukl* containing a case of kala-azar. In 9 cases smears were made from the spleen, liver and heart's blood, in 11 cases smears were made from the spleen and bone-marrow, and in one case from the spleen and liver. All were negative, no kala-azar parasites being found. We recognise that the number of dogs examined was too small to say definitely that spontaneous Leishmaniosis does not occur here in the dog. In the dog living in the infected *tukl* the spleen was enlarged, but careful search failed to reveal the presence of Leishman-Donovan bodies. Spleen smears were also examined from the spleens of 4 healthy cows, 3 healthy goats and 15 healthy sheep, all with negative results.

There is now no doubt that Leishmaniosis occurs in dogs as a natural infection, and Nicolle's discovery has been confirmed in different parts where infantile kala-azar is found. Basile recognises two different forms of natural infection in the dog, the acute and the chronic, and he considers that the former, which affects young dogs and lasts from 3 to 4 months, plays the more important part in the spread of the disease.

Kala-azar a
natural infec-
tion in dogs

TABLE I

Animal	Where inoculated and date of inoculation	Material inoculated	Course of Disease			Result	Organs infected	Remarks
Monkey B	Liver and peritoneum Jan. 9, 1910	Post mortem spleen puncture Case 4	30th day Liver puncture negative	53rd day Liver puncture negative	62nd day Chloroformed	Infected	Spleen + Liver —	Spleen slightly enlarged. Parasites present in moderate numbers
Monkey C	Peritoneum March 12, 1910	Post mortem spleen emulsion Monkey B	8th day Peripheral blood negative	33rd day Peripheral blood negative	45th day Peripheral blood negative Liver puncture negative	60th day Liver puncture negative	94th & 95th days Liver puncture negative	108th day Chloroformed
Monkey D	Peritoneum April 21, 1910	Spleen puncture Case 3	14th day Peripheral blood negative	55th day Liver puncture negative	63rd day Ill, dyspnoea, splenomegaly Liver puncture negative Spleen puncture negative	65th day Died	Spleen + Liver + Bone-Marrow +	Miliary tuberculosis also present. Spleen markedly infected with tubercle
Monkey E	Peritoneum April 23, 1910	Spleen puncture Case 1	14th day Peripheral blood negative	54th day Liver puncture positive	58th day Peripheral blood negative	78th day Spleen enlarged Spleen puncture positive Liver puncture negative Peripheral blood negative	143rd day Diarrhoea Liver puncture positive	145th day Chloroformed
Monkey H	Peritoneum June 21, 1910	Post mortem spleen emulsion Case 1			65th day Chloroformed	Not infected	Spleen — Liver — Bone-Marrow —	The spleen emulsion from Case 1 was made 8 hours after death

TABLE I—continued

Animal	Where inoculated and date of inoculation	Material inoculated	Course of Disease		Result	Organs infected	Remarks
Monkey K	Subcutaneous tissues July 11, 1910	Spleen puncture Monkey E	160th day Spleen enlarged Liver puncture negative	161st day Spleen puncture negative	163rd day Spleen puncture negative		Still alive
Monkey L	Intravenous and subcutaneous July 11, 1910	Spleen puncture Monkey E		161st day Spleen enlarged Spleen puncture positive	Infected		Still alive
Monkey N	Peritoneum July 11, 1910	Spleen puncture Monkey E	120th day Liver puncture doubtful		155th day Died	Infected Spleen + Liver + Bone-Marrow +	Small ulcer in small intestine. Smears and sections of this negative
Monkey O	Peritoneum July 26, 1910	Post mortem spleen puncture Case 10			42nd day Found dead	Not infected Spleen — Liver — Bone-Marrow —	Spleen not enlarged
Monkey P	Peritoneum July 31, 1910	Post mortem spleen emulsion Case 11	123rd day Liver puncture positive		134th day Died	Infected Spleen + Liver + Bone-Marrow +	
Monkey R	Subcutaneous tissues July 31, 1910	Post mortem spleen emulsion Case 11	150th day Spleen enlarged Liver puncture positive		156th day Peripheral blood positive	Infected	Still alive
Monkey S	Not inoculated	Kept in cage beside Monkey N	112th day Ill	117th day Liver puncture positive	121st day Died	Infected Spleen + Liver + Bone-Marrow +	Lived continuously with Monkey N, became infected and died before Monkey N

EXPERIMENTAL KALA-AZAR IN THE GREY MONKEY
(*Cercopithecus sabæus*)

Inoculation experiments were carried out to determine the infectivity of the ordinary grey monkey of the Sudan (*Cercopithecus sabæus*) to kala-azar, and the results are shown in Table I. (pages 162-163).

Twelve monkeys were used in the experiments. Eight were infected with kala-azar. In 3 the experiment failed, and one still remains doubtful. So far we have found that the best method of infecting these monkeys is from a splenic puncture during life. An ordinary sterilised hypodermic syringe is taken, washed out with sterile citrate, spleen puncture carried out in the ordinary way and the contents of the syringe immediately injected into the peritoneal cavity of a monkey.

Infected cases

Infected cases

Of the 8 infected cases, 5 were infected intraperitoneally, one subcutaneously, one subcutaneously and intravenously, and one was naturally infected.

Of those infected intraperitoneally, Monkey B was chloroformed on the 62nd day. Post mortem examination showed enlargement of the spleen and Leishman-Donovan bodies present in moderate numbers. No parasites could be found in the liver, so probably the spleen becomes infected before the liver.

Monkey D died on the 65th day from a mixed infection of tuberculosis and Leishmaniosis and it is interesting to record the degenerate nature of the parasites in this case. The parasites were most degenerated in the spleen, which was heavily infected with tubercle, and were best preserved in the bone-marrow where the tubercles were scanty. The post mortem examination was done immediately after death, so the change could not be a post mortem one.

Monkey E was infected by the 54th day, became very ill with diarrhoea on the 143rd day, and was chloroformed on the 145th day. It was heavily infected with parasites.

Monkey N, inoculated intraperitoneally from Monkey E, remained quite well, but, owing to infection occurring in Monkey S (*vide infra*), liver puncture was carried out on the 120th day and doubtful Leishman-Donovan bodies were seen; 35 days later the monkey died and the post mortem examination showed splenomegaly with Leishman-Donovan parasites present in the spleen, bone-marrow and liver. There was a small ulcer in the small intestine, in the region of the ileum.

Monkey P died on the 134th day heavily infected with Leishman-Donovan parasites.

Infection by a
subcutaneous
injection

Of those infected subcutaneously, Monkey R was inoculated in the subcutaneous tissues of the thigh with a post mortem splenic emulsion from a case of kala-azar. On the 150th day the spleen was found to be enlarged and liver puncture showed the presence of Leishman-Donovan bodies. On the 156th day the peripheral blood was examined and in the 5th slide 3 parasites were found inside a large mononuclear cell. This monkey is still alive.

Monkey L was injected intravenously and subcutaneously. It was intended to inject intravenously, but only the first portion of the virus entered the vein, the remainder entering the subcutaneous tissues of the forearm. Examined on the 161st day the spleen was found to be enlarged and splenic puncture showed the presence of Leishman-Donovan bodies. This monkey is still alive.

The remaining monkey, which was a very young animal, was put in the same cage as Monkey N on the day that the latter was inoculated intraperitoneally from Monkey E.

It remained continuously in that cage, and on the 112th day it became ill. On the 117th day liver puncture showed the presence of Leishman-Donovan bodies. It died on the 121st day, 34 days before Monkey N. It was very heavily infected, the parasites being present in large numbers in the liver, spleen and bone-marrow.

Non-infected cases

Monkey H was inoculated with a splenic emulsion from Case I., the emulsion being made 8 hours after the death of the patient, when no parasites were present in the spleen smears. (Monkey E was successfully infected from the same case during life). It was chloroformed on the 65th day, but no Leishman-Donovan parasites were found. Non-infected cases

Monkey C was inoculated with a post mortem spleen emulsion from Monkey B, but was not infected by the 108th day, when it was chloroformed. Probably, as Nicolle has pointed out, the parasite tends to lose its virulence when inoculated from monkey to monkey, and this might account for the non-infection in this case, though the positive results obtained in Monkeys L and N show that infection can be conveyed from monkey to monkey. In the two latter, however, the inoculation was made from a splenic puncture during life, and in this monkey a post mortem splenic emulsion was used.

Monkey D died 42 days after inoculation. There was no apparent cause of death, and no Leishman-Donovan parasites were found.

Monkey K, which was inoculated subcutaneously, shows definite enlargement of the spleen, but one liver puncture and two spleen punctures, have so far given negative results.

The presence of Leishman-Donovan parasites in the blood of infected monkeys

Parasites are present in the peripheral blood of infected monkeys, but are difficult to find. In two infected monkeys we have searched carefully for parasites in the peripheral blood. In one monkey we found, in the fifth slide examined, three parasites inside one large mononuclear cell, and in another monkey, out of 7 slides examined, we found one parasite. In this instance it was inside a polynuclear cell. The presence of Leishman-Donovan parasites in the blood of infected monkeys

Though dogs have been more frequently used in experimental kala-azar, Nicolle and his colleagues have frequently infected monkeys, though so far only by the intraperitoneal method, the subcutaneous method giving only a local reaction, without a general infection. They also found that passage of the disease from monkey to monkey attenuated the virus, though the same phenomenon did not apply in dogs. They consider that in the monkey the disease more resembles the human type, marked enlargement of the spleen being usually present. Our results agree with those obtained by these observers, but we have succeeded in infecting monkeys by subcutaneous inoculation, and have also naturally infected one monkey.

EXPERIMENTAL KALA-AZAR IN THE DOG

Inoculation experiments were also carried out to determine if the dog could be infected with kala-azar and the results are shown in Table II. (page 166).

TABLE II

Animal	Where inoculated and date of inoculation	Material inoculated	Course of Disease	Result	Organs infected	Remarks
Dog B ...	Liver and peritoneum March 12, 1910	Post mortem splenic emulsion Monkey B	14th day Died	Not infected	Spleen - Liver - Peritoneal fluid -	
Dog D ...	Liver and peritoneum May 27, 1910	Spleen puncture Case 1	31st day Liver puncture negative 50th day Axillary abscess Emaciation Liver puncture negative 52nd day Died	Not infected	Liver - Bone-Marrow -	Spleen smears unsatisfactory Post mortem examination some hours after death
Dog F ...	Peritoneum July 2, 1910	Liver puncture Monkey E	16th day Again inoculated intraperitoneally Spleen puncture Monkey E 19th day Died	Not infected	Spleen - Liver - Bone-Marrow -	
Dog G ...	Subcutaneous tissues July 2, 1910	Ditto	16th day Ditto 22nd day Died	Not infected	Spleen - Bone-Marrow -	
Dog H ...	Peritoneum September 13, 1910	Post mortem spleen emulsion Monkey E	7th day Died	Not infected		Phroplasmosis present

Infection of dogs was, therefore, not obtained, but it is difficult to account for the mortality. Probably the mortality in Dogs F and G, which were re-inoculated on the 16th day with the contents of a splenic puncture (Leishman-Donovan parasites not being seen in smears made from the original liver puncture with which they were inoculated), may be attributed to some anaphylactic effect of the body fluids from Monkey E. Dog D became emaciated, developed an abscess in the axilla, and we thought it would be infected, particularly as the spleen was found enlarged after death. Unfortunately, the post mortem examination was not done until some hours after death, when the spleen pulp was too soft for satisfactory examination, but the bone-marrow contained no parasites. As Dog D was the only one which lived long enough to become infected with the disease, further experiments are necessary to determine if the dog can be experimentally infected with the Sudan form of kala-azar.

A large amount of experimental work has now been done on kala-azar in the dog. Nicolle and his colleagues have frequently infected dogs. They find in dogs that the infection is an insidious one with only slight enlargement of the spleen. One of the dogs which they inoculated with the disease and which died 17 months after inoculation showed no symptoms for 15 months. They also found that in a series of dogs there was great variation in the susceptibility of the individual animal. They found no attenuation in the virus by passage from dog to dog. With regard to immunity they have found that in an animal incompletely cured or only just recently recovered, a second inoculation produces a severe and fatal result, whereas, in an animal cured for some time immunity is conferred. Excision of the spleen in the dog has produced no effect on the course of the disease.

Kala-azar in
the dog

Jemma, Gabbi, and Alvarez and Pereira da Silva, have also all succeeded in infecting dogs intraperitoneally with *Leishmania infantum*. Basile was able to infect three young dogs by keeping them beside cases of infantile kala-azar and he thinks the flea (*Pulex serraticeps* and perhaps *Pulex irritans*) is the likely transmitting agent. Jemma has also been able to infect dogs intravenously as well as intraperitoneally.

Novy, by the use of large and repeated doses of cultures of *Leishmania infantum* on Novy and MacNeal's medium, has been able to produce infection in the dog.

Patton, working with Indian kala-azar, failed to infect dogs even with repeated inoculation, but probably he killed his animals too soon to say definitely that infection does not occur.

MORPHOLOGY AND CULTURE OF THE PARASITE

The parasites in these cases do not appear to differ in structure from the parasites described in Indian kala-azar and in infantile kala-azar. The parasites were sometimes present in spleen cells and sometimes lying free. So-called developmental forms were frequently found containing up to as many as 11 nuclei. Our observations agree with those of other observers who find that, when a large amount of blood is obtained in spleen puncture, the parasites are scanty and difficult to find. There is definite evidence that the parasite degenerates quickly after death. As shown above, in one case where the parasites were found in large numbers during life the post mortem examination, 8 hours after death, showed no parasites in the spleen. In another case, examined 4 hours after death, the parasites were present but showed evidences of degeneration, the nucleus and kinetonucleus being visible, but no protoplasm.

Rapid
degeneration
of the parasites
after death

In two cases the parasites were successfully cultivated in 10 per cent. citrate. Flagellated forms were found as early as the second day, but seemed to be most numerous and most active on the 4th and 5th days. The parasites move very slowly with the

flagellum in front. The flagella are usually very active. There are other forms present, some very large, which show no tendency to develop into the flagellate form. These measure about 5μ by 6.5μ , but we saw one measuring 7.65μ by 6.8μ . We have found living parasites in these cultures on the 28th day.

Culture

The parasite also grows on Novy and MacNeal's medium and on Nicolle's modification of that medium. We obtained cultures on these media with the material from the splenic puncture of an infected monkey.

Cultures made from post mortem spleen punctures were invariably negative, even though the parasites were present in considerable numbers.

INFECTION BY THE BED-BUG (*Cimex lectularius*)

Feeding
experiments
negative

Feeding experiments with the bed-bug (*Cimex lectularius*) were carried out in Cases 1 and 3, but with negative results. So far, we have not met with the *Cimex rotundatus* in the course of our investigations. Owing to the small number of parasites found in the peripheral blood in our cases, these feeding experiments are of little value, as, in cultural experiments of the parasite, we found that the presence of a considerable number of parasites was necessary to give a positive growth into flagellate forms.

OTHER PROTOZOA MET WITH IN THE COURSE OF THE INVESTIGATION

- (a) Protozoon from post mortem splenic puncture of Case 4.
 - (b) Flagellated bodies found in water and in fæces of infected monkey.
 - (c) Flagellated bodies present in soil.
- (a) *Protozoon from post mortem splenic puncture of Case 4* (Plate X., figs. 5-7)

Cultures were made from the post mortem splenic puncture of Case 4 from which Monkey B was successfully infected with kala-azar. After 48 hours the four culture tubes were examined; three showed micro-organisms, the fourth showed micro-organisms and unaltered Leishman-Donovan parasites. Examination of this tube, 4 days later, showed the presence of large, slightly motile bodies with a tendency to become oval and, evidently, from the currents produced, ciliated. They appeared to possess amœboid properties. These bodies developed, in a short time, into oval, actively motile bodies moving always with the sharp end in front. In a hanging-drop slide ringed with paraffin they remained motile for at least 48 hours. A contractile vacuole was visible at the posterior end. Stained preparations show a definite pale area near the anterior end, sometimes on the right side and sometimes on the left. This has the appearance of a peristome. These bodies are ciliated all round but the cilia are more numerous at the anterior end, and are particularly so round the pale area. The nucleus is large but varies in shape and situation. The protoplasm is vacuolated and full of bacteria. Amœboid projections from the edge of the body are sometimes present, a small projection from the posterior end being particularly common.

This parasite appears to live indefinitely in the original fluid, though subculture on various media always gave negative results, the parasite not being able to multiply and soon dying out. Injected intraperitoneally, it was non-pathogenic for a young dog. The parasite was able to live for some days in media containing blood, but did not multiply. The younger stage of this parasite is seen as a small, circular, deeply-stained nucleated body occurring for the most part in groups. Whether these

Subculture
unsuccessful

are the result of encystment and rupture, or whether they are budded off from the parent body, we cannot tell. These small round bodies develop into small oval bodies which gradually increase in size, becoming in time a full-grown parasite. The size of a full-grown parasite is about 42.5μ by 34μ .

This parasite evidently belongs to the *Balantidium* group of protozoa, but does not conform to the description given of either of the two described as occurring in the human alimentary canal. It is smaller than *Balantidium coli*, and differs also in having only one nucleus and one contractile vacuole. It is bigger than the *Balantidium minutum* of Schaudinn, and differs also in the structure of the peristome. The special aggregation of the cilia at the anterior or pointed end is also characteristic of this new form.

Balantidia are usually associated in man with pathological conditions, producing enteritis and sometimes ulceration of the bowel. It is an interesting fact that it was obtained by splenic puncture from a case of kala-azar which had suffered from diarrhoea. It is also interesting to record that this parasite was again obtained in a culture made from the mucous membrane around the ulcer in the large intestine of Case 11.

(b) (1) *Flagellated bodies in standing water* (Plate X., figs. 8 and 9)

This parasite was found accidentally while we were endeavouring to cultivate flagellated bodies from the faeces of infected monkeys. The control of ordinary distilled water showed in three to four days an enormous number of very actively motile bodies. These bodies are small, oval or circular, and, in stained preparations, appear to have usually two flagella. The longer flagellum comes from the region of the nucleus and the shorter one from the kinetonucleus. The protoplasm is vacuolated. The average measurement is 6.8μ by 5μ . The flagellum is usually longer than the parasite. On examining the original bottle containing the distilled water a few parasites were found. These evidently multiply rapidly at 22°C .

Flagellated
bodies in
standing water

(b) (2) *Flagellated bodies in faeces of infected monkey* (Plate X., figs. 10-12)

These were obtained by emulsifying the faeces of Monkey E in boiled distilled water and incubating at 22°C . These bodies very much resemble the preceding, but are smaller and more elongated. Sometimes one flagellum was present and sometimes two, the latter being the more common. Usually the two flagella arise quite close together, and, in many parasites, the kinetonucleus is not visible. The average length of the parasite is about 6μ by 3μ . Further experiments are necessary to determine if these parasites are present in the faeces of other infected monkeys, controls with healthy monkeys so far having given negative results.

Flagellated
bodies in faeces
of infected
monkey

(c) *Flagellated bodies in soil*

Enormous numbers of flagellated bodies were obtained from soil, but their morphology has not yet been worked out.

Flagellated
bodies in soil

CONCLUSIONS

1. Kala-azar in Sennar Province affects, for the most part, children whose average age is about 12 years. This does not appear to hold in the neighbouring Province of Kassala where adults are commonly affected.
2. The disease runs an acute course, and chronic cases were not met with.
3. The disease is not invariably fatal, cases of recovery having been met with.

4. The Leishman-Donovan parasite was found in the peripheral blood in 86.6 per cent. of the cases.
5. The ordinary grey monkey of the Sudan (*Cercopithecus sabæus*) can be infected with kala-azar.
6. It can be infected by intraperitoneal inoculation and by subcutaneous inoculation.
7. Natural infection, from an infected to a healthy animal, can occur provided the animals are kept in close contact.
8. The Leishman-Donovan parasite is present in the peripheral blood of infected monkeys.
9. So far we have found that the best method of infecting the monkey is by injecting into the peritoneal cavity the contents of a spleen puncture taken during life.
10. So far we have not succeeded in infecting dogs nor have we found spontaneous Leishmaniosis in the dog.
11. The parasite can be cultivated readily on artificial media, development into flagellate forms being obtained in 10 per cent. citrate, on Novy and MacNeal's medium and on Nicolle's modification of that medium.
12. The parasite appears to degenerate quickly after the death of the host. Post mortem examinations and post mortem spleen punctures for diagnostic purposes should be done immediately after death.

POSSIBLE METHODS OF INFECTION IN KALA-AZAR

From the evidence obtained it appears likely that some insect acts as the transmitting agent of kala-azar in the Sudan. You can take unaltered parasites from one monkey, inject them subcutaneously into another monkey, and the latter becomes infected with the disease, so that presumably the disease can be conveyed without any extracorporeal development into the flagellate stage. Further, a healthy monkey kept beside an infected monkey can contract the disease, and in the case recorded in this paper the infection was a rapid and fatal one, and was contracted from a monkey which was not heavily infected with the disease. How then did this monkey become infected? If an insect, the bed-bug (*Cimex lectularius*) is unlikely, as these monkeys were kept in a metal cage and no bed-bugs were found, so that some other insect transmitter must be looked for, and further experiments will be carried out to elucidate this point. Possibly also, if an insect is the transmitting agent, only a small percentage of the feeding or biting animals become infected (owing to the small number of parasites in the peripheral blood) and this would account for the absence of epidemics and the apparently wide-spread distribution of the disease. The presence of a flagellate form also suggests an intermediate host and a developmental stage outside the human body.

Probability of
an "insect"
vector

The other possible source of infection is an intestinal one, the flagellate form occurring free outside the body and entering the body again by water or by food. If so, in the naturally infected monkey, the monkey must have eaten food contaminated by the excreta of the infected monkey.

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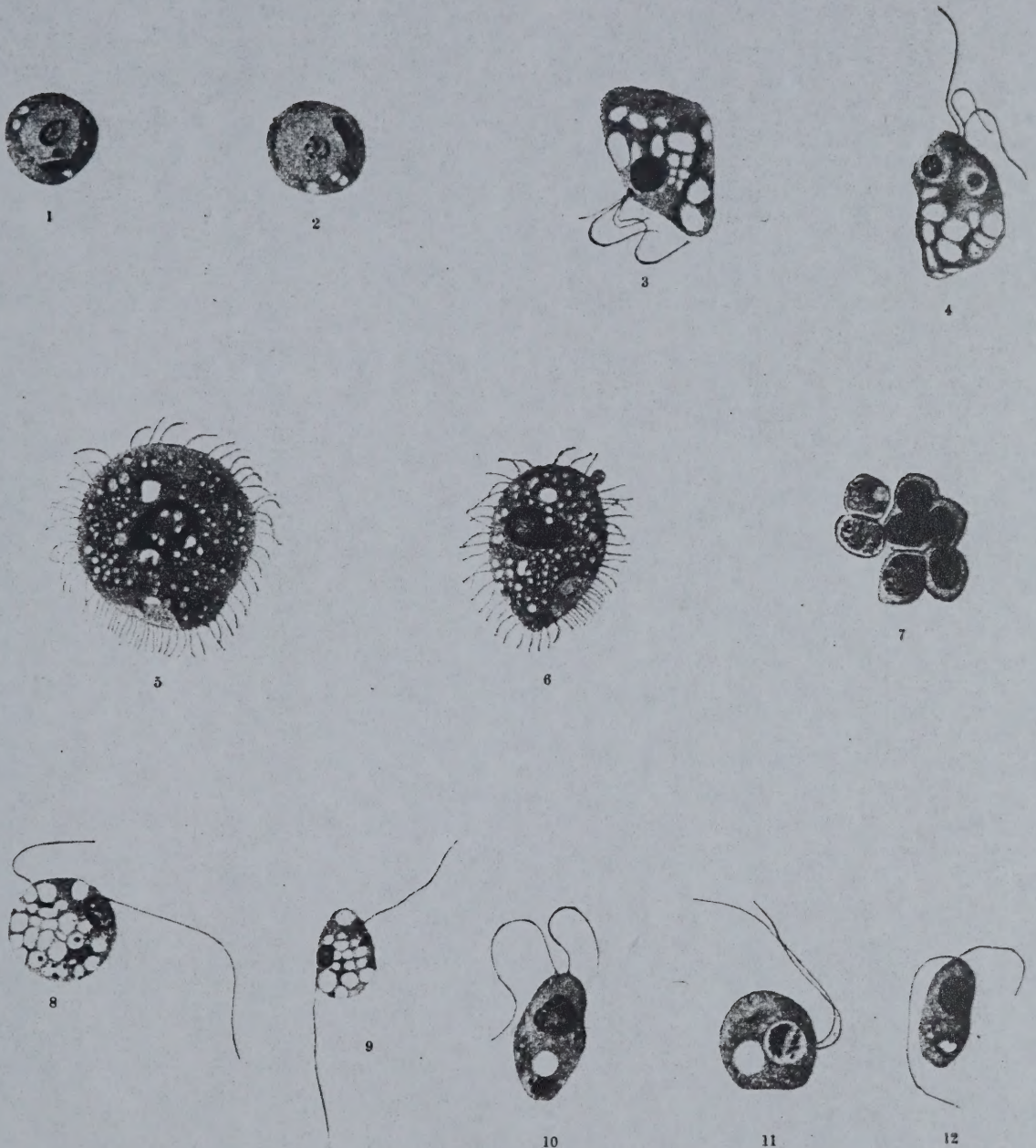
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PLATE X



M. RHODES

1-4, $\times 1000$ diam.; 5-6, $\times 800$ diam.; 7, $\times 1500$ diam.; 8-12, $\times 2000$ diam.

PROTOZOA OTHER THAN KALA-AZAR MET WITH BY THE COMMISSION

- 1-2. Cyst-like bodies from faeces, case of kala-azar. Probably one stage in figs. 3 and 4.
- 3-4. Trichomastix bodies from same case.
- 5-6. Balantidium found in two cases, from spleen puncture and from the mucous membrane of the large intestine. Adult parasite.
7. Young forms of 5 and 6.
- 8-9. Flagellated bodies in distilled water.
- 10-12. Flagellated bodies from faeces of monkey infected with kala-azar.

